# PROTOCOL AND STATISTICAL ANALYSIS PLAN

# NATURAL COURSE OF SUBCLINICAL HYPERTHYROIDISM IN PRIMARY CARE IN THE NETHERLANDS

**Study design and setting**

We will conduct a retrospective cohort study using data from the Dutch General Practitioner (GP) Database from the PHARMO Data Network. The GP Database is a longitudinal database comprising data from electronic patient records registered by GPs. Records include information on diagnoses and symptoms, laboratory test results, and prescriptions. Prescription records include information on the type of product, prescription date, strength, dosage regimen, quantity, and route of administration. Drug prescriptions are coded according to the WHO Anatomical Therapeutic Chemical (ATC) Classification System. Diagnoses and symptoms will be coded according to the International Classification of Primary Care (ICPC). The GP Database covers a catchment area representing 3.2 million residents (~20% of the Dutch population). More information has been published elsewhere (Overbeek JA, Swart KMA, Houben E, Penning-van Beest FJA, Herings RMC. Completeness and Representativeness of the PHARMO General Practitioner (GP) Data: A Comparison with National Statistics. Clin Epidemiol. 2023;15:1-11. https://doi.org/10.2147/CLEP.S389598)

**Time frame**

Data from January 1, 2012, to December 31, 2021, will be analyzed, encompassing a ten-year period to assess the incidence trends of subclinical hyperthyroidism (SHT) and its natural course.

**Incidence**

For the incidence, all people of 18 years and older in the PHARMO database who were registered as being present in the GP practice (active) on January 1st of each calendar year and who were registered at least one year in the patient file will be included. All with SHT before January 1st of each calendar year are excluded. Additionally, patients using lithium (anywhere before SHT), amiodarone or thyroid medication (2 years before SHT) will be excluded. The observed incidence of SHT will be determined among all people in the patient file who had at least one year of data available, defined as the population at risk. At least one year of data availability is required to ensure each person have an equal chance of being marked as an incident person with SHT. The observed incidence will be calculated by dividing the sum of people with incident SHT by the total number of person-time at risk in the patient file for both females and males during the calendar year. Person-time at risk in the patient file is defined as the time between January 1st of the year, or start follow-up in the GP practice, whatever came last, and diagnosis date of SHT, December 31st of the year or end of follow-up in the GP practice, whichever came first.

**Study population for dataset**

The study population will consist of patients identified through TSH measurements requested in primary care, excluding those with known thyroid diseases.

Subclinical hyperthyroidism is defined by a TSH concentration below the lower limit of the reference interval and an FT4 concentration within the reference interval at the same measurement or patients with a recorded ICPC code A91.07 (subclinical hyperthyroidism). Method specific reference intervals will be used for each TSH and FT4 measurement.

Exclusion criteria for subclinical hyperthyroidism are: in the 2 years prior to inclusion (1), use of thyroid medication (ATC starting with H03), amiodarone (ATC C01BD01) or ever recorded use of lithium (ATC N05AN01) or (2) mention of ICPC codes: T85 (hyperthyroidism), T86 (hypothyroidism), A91.06 (subclinical hypothyroidism), A91.07 (subclinical hyperthyroidism) or T71 (thyroid malignancy).

The patients included based on ICPC code could include several misclassifications, where the biochemical diagnosis was subclinical hypothyroidism, in that case, only patients with a laboratory confirmed subclinical hyperthyroidism will be included. Furthermore, patients without available data for the study period or who could not be matched to controls will be excluded from the analysis. In addition, subjects younger than 18 years of age at time of inclusion will also be excluded. In order to minimize the chance of thyroid disorders being related to pregnancy we will exclude patients with subclinical hyperthyroidism which are included three months before to one year after a registered pregnancy (ICPC codes W78 "Desired pregnancy" or W79 "Unwanted pregnancy"). If a pregnancy is registered in the follow-up TSH and FT4 values will also be excluded in the lab dataset three months before to 1 year after a registered pregnancy. We will perform a sensitivity analysis, requiring a second suppressed TSH measurement 4 weeks to 6 months after the initial TSH measurement for inclusion as subclinical hyperthyroidism. A detailed overview of the patient selection process will be presented in order to illustrated the flow diagram of the inclusion and exclusion criteria applied in this study.

**Exploratory and confirmatory factor analysis**

Exploratory factor analysis (EFA) was conducted on a randomly selected subset comprising 25% of the database. The EFA helped to define the exact variables and develop robust outcome measures. After finalizing the analysis protocol based on the EFA results, the protocol was registered on the ISRCTN registry to maintain transparency and reproducibility.

Once the analysis protocol is published, the remaining 75% of the database, which had been withheld to prevent bias, will be decoded and made available for research. A confirmatory factor analysis (CFA) will then be performed on this larger dataset to validate the factors identified in the EFA and to confirm the consistency and reliability of the defined outcome measures.

**Outcome measures**

In this study, we will identify the following subgroups:

1. Progression to overt hyperthyroidism, defined as FT4 levels above the upper limit of the reference interval during the follow-up period.
2. Progression to (subclinical) hypothyroidism, defined as a TSH level above the upper limit of the reference interval at any time during follow-up, but not in group 1.
3. Recovery, characterized by TSH levels returning to within the reference interval at any time during the follow-up, but not in group 1 or 2.
4. Persisting subclinical hyperthyroidism, defined as persistently suppressed TSH levels with FT4 levels within the reference interval throughout the follow-up period, but not in group 1-3.

5. Unknown, which group includes patients who were not included in the other groups due to missing TSH or FT4 measurements during follow-up.

For the above groups the TSH and FT4 concentrations in the first four weeks will be excluded, since they were deemed too close to the inclusion date and the Dutch primary care guideline recommends testing only after three months. We will assess the time to group definition, age, sex and TSH concentrations at inclusion. Additionally, we will examine which patients started using thyromimetics (ATC codes starting with H03A) or antithyroid drugs (ATC codes starting with H03B). These comparisons will be made across the entire available follow-up period, as well as with cut-off points at 2 years and 5 years.

Patients who initiated antithyroid therapy will also separately be examined on age, sex, TSH levels at inclusion, total follow-up time, and thyromimetic use. We will perform a sensitivity analysis for the recovery group in order to explore the amount of patients that relapsed after being marked as recovered and excluding those from the analysis.

**Guideline adherence**

We will assess guideline adherence to the Dutch primary guideline by examining the frequency with which thyroid function tests (TSH and FT4) are performed during the follow-up period. Thyroid function tests in the first four weeks will be excluded as they were deemed too close to inclusion date. Furthermore, we will evaluate adherence to recommended diagnostic workups, such as the measurement of TSH receptor antibodies, erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), and leukocyte counts. Additionally, we will investigate the occurrence of non-recommended diagnostic tests, including TPO antibody measurements.

**Statistical analysis**

To compare subgroups, we will utilize ANOVA for continuous variables and Chi-square tests for categorical variables. To identify factors associated with progression or recovery, we will perform logistic regression analyses. The dependent variables in these models are recovery versus persisting subclinical hyperthyroidism and progression versus persisting subclinical hyperthyroidism. The independent variables include age, sex, TSH levels at inclusion and comorbidity score. The comorbidity score will be defined as a composite variable representing the sum of individual risk factors associated with cardiometabolic disease. Each identified risk factor contributes one point to the total cardiometabolic risk score. The risk factors will be extracted for each case for one year before cohort entry date. They are as follows:

1. Hypertension: Defined by the presence of ICPC codes H86 or H87 or the use of antihypertensive medications (specifically antihypertensives [ATC C02], diuretics [ATC C03], beta-blocking agents [ATC C07], calcium channel blockers [ATC C08] or agents acting on the renin-angiotensin system [ATC C09]), whichever comes first: 1 point.
2. Hypercholesterolemia: Defined by either LDL cholesterol level >2.6 mmol/L (measurement timepoint to be determined) or use of statins (ATC C10), whichever comes first: 1 point.
3. Kidney Disease: Defined by either: estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 $m^2$ or albumin-to-creatinine ratio (ACR) >3 mg/mmol, whichever comes first: 1 point.
4. Diabetes Mellitus: Defined by the presence of ICPC code T90 or the use of diabetes medications (ATC A10), whichever comes first: 1 point.

All statistical analyses will be conducted using R version 4.2.2 (2022-10-31 ucrt). All statistical analyses were conducted using R version 4.2.2 (2022-10-31 ucrt). The following R packages were used for the analysis: car, dplyr, ggplot2, lubridate, multcomp, readr, and tidyr.